# 97-kDa Linear IgA Bullous Dermatosis (LAD) Antigen Localizes to the Lamina Lucida of the Epidermal Basement Membrane

Akira Ishiko,* Hiroshi Shimizu,* Takuji Masunaga,* Takashi Hashimoto,* Marian Dmochowski,* Fenella Wojnarowska,† Balbir S. Bhogal,‡ Martin M. Black,‡ and Takeji Nishikawa*

*Department of Dermatology, Keio University School of Medicine, Tokyo, Japan; †Department of Dermatology, Churchill Hospital, Oxford; and ‡St. John's Institute of Dermatology, London, U.K.

**Linear IgA bullous dermatosis (LAD) is an autoimmune blistering disease in which IgA autoantibodies develop against the epidermal basement membrane zone. Target antigens of the circulating autoantibodies are thought to be heterogeneous, and their ultrastructural localization has not been fully elucidated. Previous studies with immunoblotting have demonstrated that the 97-kDa autoantigen is detected most frequently in patients' sera and is thought to be a major LAD antigen. Although a recent report suggests that the 97-kDa antigen localized to the hemidesmosomal plaques and the adjacent lamina lucida, discrepancies still exist among previous immunoelectron microscopic findings.**

**To identify the precise localization of the 97-kDa LAD antigen, we used two different low-temperature immunoelectron microscopic techniques. For immunolabeling, we selected five LAD sera that had a high titer of autoantibodies against the 97-kDa LAD antigen. A post-embedding method with cryofixation and freeze substitution failed to immunolabel the 97-kDa LAD antigen. Cryoultramicrotomy with immunoelectron microscopy succeeded in preserving the antigenicity of the 97-kDa LAD antigen. In all cases, the majority of labeling occurred in the lamina lucida beneath the hemidesmosomes. No specific labeling was observed in the hemidesmosomal attachment plaques or the lamina densa or sublamina densa region, including anchoring fibrils. These results indicate that the 97-kDa LAD antigen is a component of the lamina lucida.** ***Key words: immunoelectron microscopy/bullous disease/autoimmune disease/immunogold. J Invest Dermatol 106:739–743, 1996***

Linear IgA bullous dermatosis (LAD) is an autoimmune subepidermal blistering skin disease in which the linear deposition of IgA at the epidermal basement membrane zone of the patient's skin is the principle diagnostic criterion (Chorzelski *et al*, 1987). Circulating IgA autoantibodies against the epidermal basement membrane zone are detected in some adult cases and most pediatric cases (Wojnarowska *et al*, 1988). The target autoantigens are thought to be heterogeneous based on the following observations. Indirect immunofluorescence using 1 M NaCl split skin demonstrated that most LAD sera react with the epidermal side (Willsteed *et al*, 1990) but some react with the dermal side (Dmochowski *et al*, 1993). From immunoblotting studies, several autoantigens have been proposed, including 97-kDa (Zone *et al*, 1990), 285-kDa (Wojnarowska *et al*, 1991), and 255-kDa (Dmochowski *et al*, 1993) proteins. Among them, the 97-kDa protein, which localizes to the epidermal side of the salt split skin, is detected most frequently and is thought to be a major LAD antigen (Dmochowski *et al*, 1993).

The ultrastructural localization of the LAD antigens has been investigated with several methods of immunoelectron microscopy both *in vivo* (Yaoita and Katz, 1976, 1977; Pehamberger *et al*, 1977; Dabrowski *et al*, 1978, 1979; Lawley *et al*, 1980; Yamasaki *et al*, 1982; Horiguchi *et al*, 1986; Petersen *et al*, 1986; Bhogal *et al*, 1987; Prost *et al*, 1989; Onodera *et al*, 1990; Legrain *et al*, 1991) and *in vitro* (Pehamberger *et al*, 1977; Yaoita and Katz, 1977; Horiguchi *et al*, 1986; Bhogal *et al*, 1987; Burge *et al*, 1988). *In vivo* deposition of IgA has been localized to the lamina lucida (Yaoita and Katz, 1976, 1977; Dabrowski *et al*, 1978; Lawley *et al*, 1980; Horiguchi *et al*, 1986; Petersen *et al*, 1986; Onodera *et al*, 1990; Legrain *et al*, 1991), sublamina densa (Yaoita and Katz, 1976, 1977; Pehamberger *et al*, 1977; Dabrowski *et al*, 1979; Lawley *et al*, 1980; Bhogal *et al*, 1987), or both, forming a mirror image pattern using pre-embedding immunoperoxidase electron microscopy (Yamasaki *et al*, 1982; Prost *et al*, 1989). Using a pre-embedding immunogold technique, IgA deposition has been localized to the uppermost part of the lamina lucida or within both the lamina densa and the anchoring plaques (Karpati *et al*, 1992). *In vitro* binding sites of LAD autoantibodies have been localized to the lamina lucida (Yaoita and Katz, 1977) or basilar surface of hemidesmosome (Horiguchi *et al*, 1986). Thus, LAD antigens are thought to be heterogeneous.

Recently, Haftek *et al* (1994) reported on the basis of post-embedding immunogold electron microscopic findings that the autoantibodies against the 97-kDa LAD antigen bind to both the hemidesmosomal plaque and the lamina lucida. However, this finding was not completely consistent with the previous reports

Manuscript received July 10, 1995; revised October 17, 1995; accepted for publication November 8, 1995.

Reprint requests to: Dr. Akira Ishiko, Department of Dermatology, Keio University School of Medicine, 35 Shinanomachi, Shinjuku, Tokyo 160, Japan.

Abbreviation: BP, bullous pemphigoid.

0022-202X/96/$10.50 

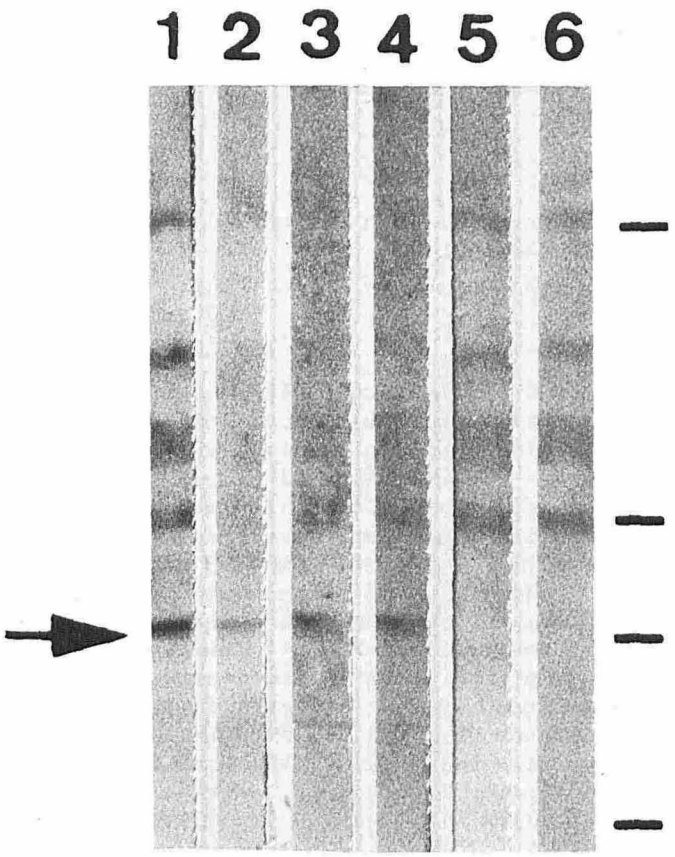


**Figure 1. LAD sera reacted with the 97-kDa epidermal protein.** Immunoblotting of the epidermal extracts with LAD sera. *Bars*, the positions of the following molecular weight markers (from top to bottom): 200, 116, 97, and 66 kDa. *Lanes 1* through *4*, LAD sera reacting with the 97-kDa protein; *lanes 5* and *6*, control normal sera producing nonspecific bands which were also produced by some LAD sera. The 97-kDa protein is not recognized by the normal control sera.

(Yaoita and Katz, 1977; Horiguchi *et al*, 1986) or with our preliminary observation using the same method.

The purpose of this study was to investigate the hitherto controversial ultrastructural localization of the 97-kDa major LAD antigen. We selected five LAD sera reactive with the 97-kDa antigen and used two currently established immunoelectron microscopic techniques: (i) a post-embedding method with cryofixation and freeze substitution, and (ii) cryoultramicrotomy.

## MATERIALS AND METHODS

**Characterization of LAD Sera** Of 46 LAD sera available, we used five sera for immunoelectron microscopy. Immunoblot was performed as previously described (Dmochowski *et al*, 1993) to select LAD sera that recognized the 97-kDa protein of the human epidermal extract. Indirect immunofluorescence on normal human skin was carried out to determine the titer of IgA anti-epidermal basement membrane autoantibodies. Indirect immunofluorescence on 1 M NaCl split skin was performed as described previously (Gammon *et al*, 1984). Normal human sera, bullous pemphigoid (BP) serum that only recognized the 230-kDa BP antigen, and BP serum that only recognized the 180-kDa BP antigen (Ishiko *et al*, 1993) were used as controls.

**Immunoelectron Microscopy** *Postembedding Immunogold Method with Cryofixation and Freeze Substitution:* Postembedding immunogold method was performed as described previously (Shimizu *et al*, 1989, 1989; Ishiko *et al*, 1993). Briefly, normal human skin was cryofixed by plunging into liquid propane (−190°C), freeze substituted with methanol for 48 h or acetone for 120 h at −80°C, and embedded in Lowicryl K11M (Chemische Werke Lowi, Waldkraiburg, Germany). Ultrathin sections were cut and incubated with LAD sera diluted 1:10. They were then incubated with rabbit anti-human IgA $(Fab')_2$ (1:500 dilution; DAKOPATTS, Copenhagen, Denmark) or mouse anti-human IgA (1:20 dilution, DAKOPATTS), followed by incubation with 5 nm gold-conjugated goat anti-rabbit immunoglobulins (1:40 dilution; Amersham International, Amersham, Buckinghamshire, U.K.) or 5 nm gold-conjugated goat anti-mouse immunoglobulins (1:40 dilution, Amersham International), respectively.

As a positive control, BP sera were used as primary antibodies and followed by incubation with 5 nm gold-conjugated anti-human IgG (Amersham International). As a negative control, normal human sera were used instead of LAD sera.

*Immunogold Labeling on Cryoultrathin Sections:* Cryoultrathin sections of normal human skin were obtained basically according to the method described by Tokuyasu (1985), with some modification. Fresh normal human skin was cut into small pieces (<1 $mm^3$) and immersed in 2.3 M sucrose in phosphate buffer (pH 7.4) with or without prefixation by ethanol at 4°C for 30 min. The samples were mounted on pins, rapidly frozen by plunging into liquid propane (−190°C), and stored in liquid nitrogen until use. Semithin sections were cut from the surface of the samples and stained with toluidine blue. Ultrathin sections were cut on an Ultracut S ultramicrotome with fetal bovine serum (FCS) cryosystem (Reichert-Jung, Vienna, Austria) then transferred to Formvar-coated nickel grids. After being washed with phosphate-buffered saline, the sections were incubated with 5% normal goat serum in washing buffer for 15 min. They were incubated with LAD sera (diluted 1:10) for 1 h and washed with washing buffer. They were then incubated with mouse monoclonal antibody to human IgA diluted 1:20 (DAKOPATTS), washed with washing buffer, followed by incubation with 5 nm gold-conjugated goat anti-mouse IgG (1:40 dilution, Amersham International) for 1 h. After washing, the sections were fixed with 2% glutaraldehyde for 10 min, and counterstained with uranyl acetate.

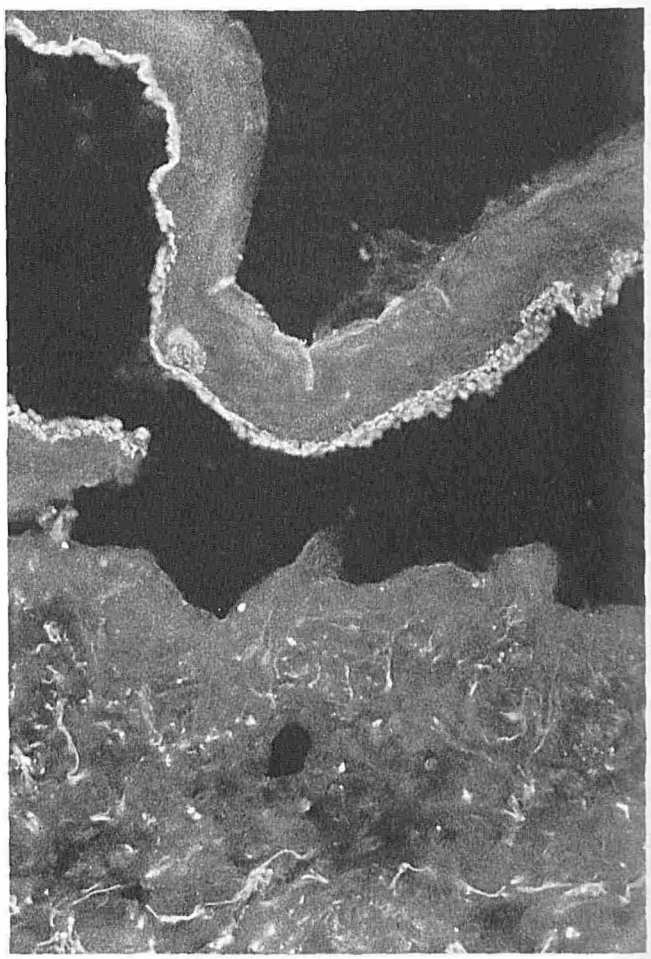

**Figure 2. LAD sera reacted with the epidermal side of the salt split skin.** Immunofluorescence of LAD sera using 1 M NaCl split human skin. All five sera used in the study bound linearly to the epidermal side of the split skin.

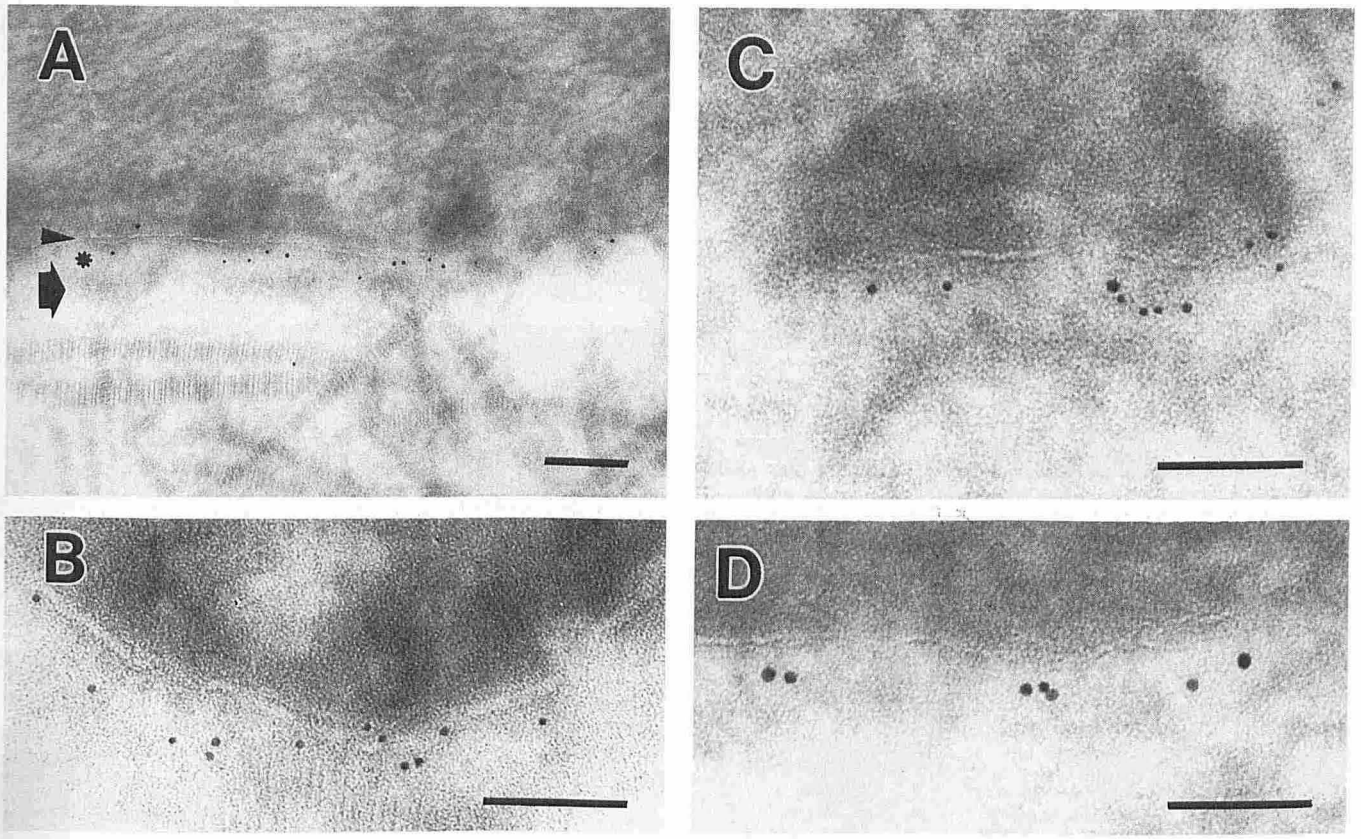


**Figure 3. Four of the five LAD sera bound to the lamina lucida in the ethanol fixed skin.** Immunoelectron microscopy using cryoultramicrotomy. Cryoultrathin sections of ethanol-fixed skin were incubated with LAD sera, followed by incubation with mouse monoclonal antibody to human IgA and 5 nm gold-conjugated anti-mouse immunoglobulins. Specific labelings were obtained in four of five cases that mainly localized to the lamina lucida associated with hemidesmosomes. *A*) Case 1. *B*) Case 2. *C*) Case 3. *D*) Case 4. ➤, plasma membrane; *lamina lucida; ⧫, lamina densa. *Scale bars*, 100 nm.

The sections were finally embedded in polyvinyl alcohol containing uranyl acetate and observed under electron microscope. As a positive control, BP sera were used as primary antibodies, followed by 5 nm gold-conjugated anti-human IgG (1:40 dilution, Amersham International) as secondary antibody. As a negative control, normal human serum was used instead of LAD sera.

## RESULTS

**Characterization of the LAD Sera for Immunoelectron Microscopy** Of the 46 LAD sera, we found 12 that reacted with the 97-kDa protein. Although they also produced several undefined bands, these were also produced by control human sera **(Fig 1)**. From the 12 sera, we further selected five that have high titer (>1:20) of IgA anti–basement membrane autoantibodies on normal human skin. Indirect immunofluorescence on 1 M NaCl split skin demonstrated that all five sera reacted with only the epidermal side but not the dermal side of the specimen **(Fig 2)**.

**97-kDa LAD Antigen is a Component of the Lamina Lucida**
*Postembedding Immunogold Method:* Ultrastructural preservation of the skin was satisfactory as previously reported (Shimizu *et al*, 1989, 1992; Ishiko *et al*, 1993). Control BP sera demonstrated a typical labeling pattern as we described previously (Ishiko *et al*, 1993); that is, the 230-kDa BP antigen was localized to the intracellular portion of the hemidesmosome and the 180-kDa BP antigen was localized along the plasma membrane of the hemidesmosome (data not shown). In contrast, none of the five LAD sera we used demonstrated any specific labeling in the skin specimens from three different individuals, probably due to loss of antigenicity.

*Immunogold Labeling on Cryoultrathin Sections:* Ultrastructural preservation was better in ethanol-fixed skin than in unfixed skin for immunogold labeling on cryoultrathin sections. However, tonofilaments, hemidesmosomes, lamina lucida, anchoring filaments, lamina densa, and anchoring fibrils were clearly observed in a small area of unfixed skin. One of five sera failed to label the ultrathin section of ethanol fixed skin, but all of the five sera demonstrated specific labeling of unfixed skin. In both fixed and unfixed skin, the labeling pattern was identical: the gold particles were mainly observed in the lamina lucida and no specific labeling was demonstrated in the attachment plaques of hemidesmosomes, or the lamina densa or sublamina densa zone, including anchoring fibrils **(Figs 3, 4)**. The distribution of the gold particles in the lamina lucida was denser beneath the hemidesmosomes compared with the nonhemidesmosomal region.

Control BP sera showed distinct labeling pattern that was similar to that observed with post-embedding immunoelectron microscopy. The BP serum reactive with the 230-kDa BP antigen labeled the intracellular portion of hemidesmosomes **(Fig 5*A*)**. However, control BP serum reactive with the 180-kDa BP antigen labeled along the plasma membrane of the hemidesmosome complex both intra- and extracellularly **(Fig 5*B*)**. Normal human sera demonstrated no specific labeling.

## DISCUSSION

We have demonstrated that LAD autoantibodies reactive with the 97-kDa major antigen bound mainly to the lamina lucida of the basement membrane of normal human skin. The 97-kDa antigen, the most detectable antigen of the epidermal LAD antigens, can be extracted by washing epidermis, separated from dermis at lamina lucida level, with Tris buffer, sodium dodecyl sulfate, mercaptoethanol, and phenylmethylsulfonyl fluoride (Zone *et al*, 1990). Its extractibility may indicate that the 97-kDa antigen localizes mainly to the lamina lucida rather than to the hemidesmosome complex.

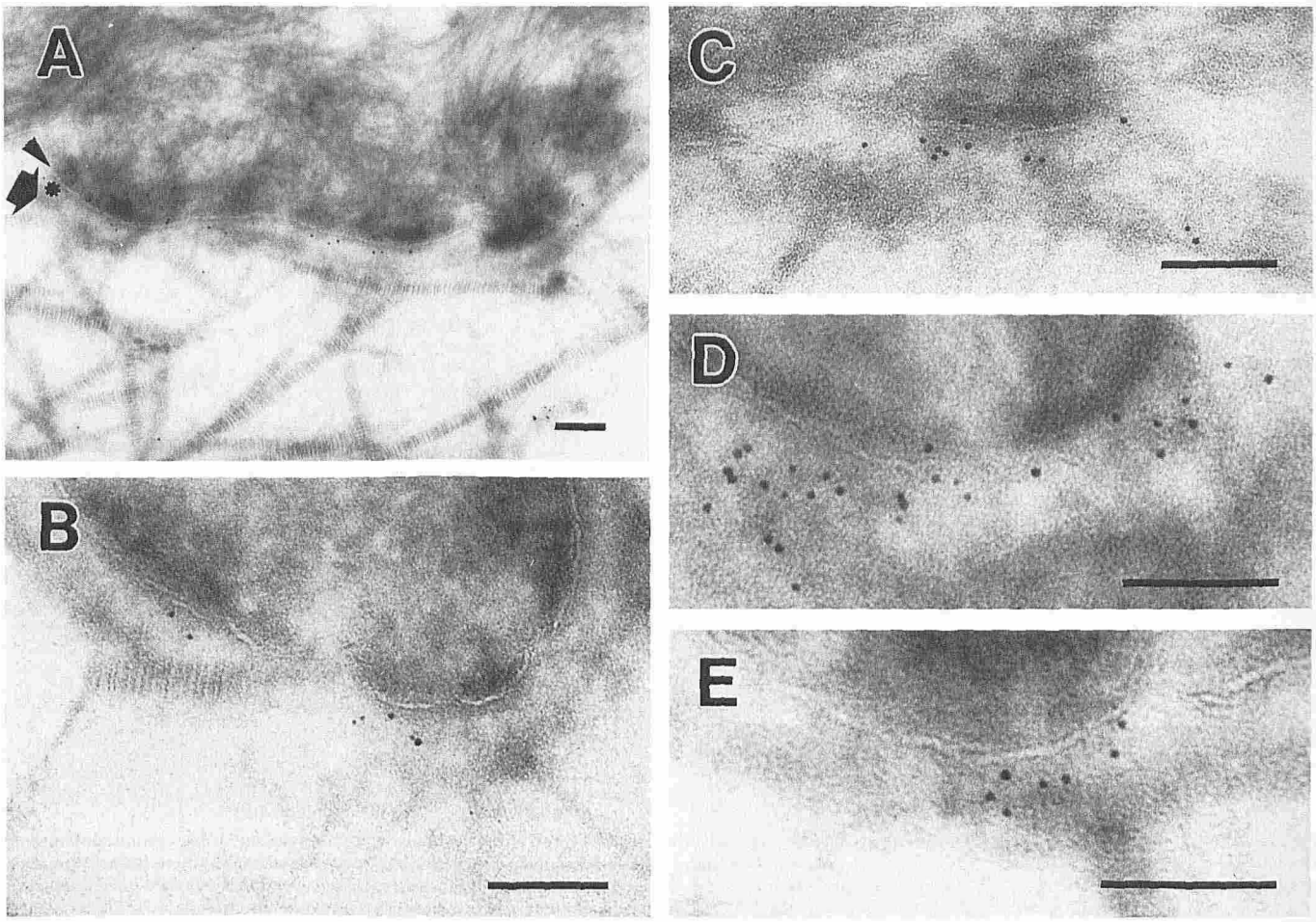


**Figure 4. All the five LAD sera bound to the lamina lucida in the unfixed skin.** Immunoelectron microscopy using cryoultramicrotomy. Cryoultrathin sections of unfixed skin were incubated with LAD sera followed by incubation with mouse monoclonal antibody to human IgA and 5 nm gold-conjugated anti-mouse immunoglobulins. In all the cases, including Case 5, labeling of gold particles were mainly observed in the lamina lucida in a pattern identical to that in ethanol-fixed skin. *A*) Case 1. *B*) Case 2. *C*) Case 3. *D*) Case 4. *E*) Case 5. ➤, plasma membrane; *lamina lucida; ⧫, lamina densa. *Scale bars*, 100 nm.

One of five LAD sera reacted with the 97-kDa antigen by present immunoblot technique were previously shown to react with 285-kDa antigen by different immunoblot technique (Wojnarowska *et al*, 1991). With the present immunoblot technique, none of the five sera including this serum only reacts with the 97-kDa band and not with the 285-kDa band. Although the reason of this discrepancy is not clear, a recent study (Wojnarowska *et al*, 1995) pointed out that the different immunoblot results were obtained by different techniques.

The ultrastructural localization of LAD antigens remains controversial. In the epidermal type of LAD, the IgA deposition in patients' skin has been reported to be localized to the lamina lucida (Yaoita and Katz, 1976, 1977; Lawley *et al*, 1980; Yamasaki *et al*, 1982; Onodera *et al*, 1990; Legrain *et al*, 1991), entire lamina lucida (Dabrowski *et al*, 1978), lower lamina lucida (Petersen *et al*, 1986), or basilar surface of hemidesmosome (Horiguchi *et al*, 1986). However, none of these reports used LAD serum characterized by immunoblot, hence their results might represent heterogeneous LAD antigens.

Recently, Haftek *et al* (1994) attempted to immunolocalize the LAD antigen using well-characterized sera that recognize the 97-kDa LAD antigen. They used our original method of low-temperature immunoelectron microscopy with cryofixation and freeze substitution (Shimizu *et al*, 1989, 1992; Ishiko *et al*, 1993) and demonstrated that the 97-kDa LAD antigen localized to both the hemidesmosomal plaque and the lamina lucida. However we had determined that none of our five LAD sera demonstrated specific labeling via the identical immunoelectron microscopic method. Because the 230-kDa BP antigen and the 180-kDa BP antigen, but not 97-kDa LAD antigen, could be immunolabeled on the ultrathin skin substrate produced by this technique, we think that the antigenicity of the 97-kDa antigen is not preserved with this procedure. We have experienced the same problems with this technique on the other anchoring filament proteins recognized by antibodies including GB 3 (Verrando *et al*, 1991) and 19-DEJ-1 (Fine *et al*, 1989). The other possibility is that the sera used in the report by Haftek *et al* (1994) recognized other epitopes in the same 97-kDa LAD antigen whose antigenicity may be preserved by the Lowicryl embedding procedure. The reason for the differences in the ultrastructural location of the 97-kDa LAD antigen between our studies and the results of Haftek remains unclear.

With the post-embedding method, antibodies as well as gold probes did not permeate to the entire thickness of the ultrathin section but bound only to the surface of the section. On the other hand, with cryoultramicrotomy, they permeated to the section and bound well to the antigens in loose structures such as lamina lucida. Therefore, we feel that cryoultramicrotomy can provide better results in the study of certain lamina lucida antigens.

Although the molecular nature and function of the 97-kDa antigen is still uncertain, our results indicate that the autoantibodies

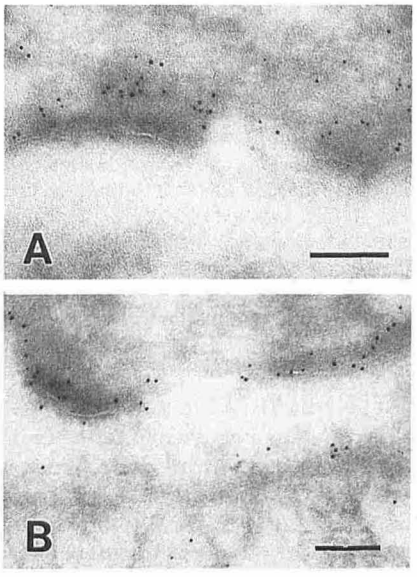


**Figure 5. Control BP sera reacted with hemidesmosomes.** Immunoelectron microscopy of control BP sera using cryoultrathin sections. *A*) BP serum reactive with the 230-kDa BP antigen labeled intracellular portion of hemidesmosomes. *B*) BP serum reactive with the 180-kDa BP antigen labeled along the plasma membrane of hemidesmosomes. Both extra- and intracellular labelings were observed. Both BP sera revealed no specific labeling at the lamina lucida as observed in all of the LAD sera. *Scale bars*, 100 nm.

against this antigen mainly bind to the lamina lucida beneath the hemidesmosomes, but not to the attachment plaques of hemidesmosomes. Based on this localization, the 97-kDa LAD antigen may be a distinct component of lamina lucida, probably related to the anchoring fibril.

*This work was supported by a Grant-in-Aid for Encouragement of Young Scientists (05770640) from the Minister of Education, Science, and Culture of Japan, and a Collaborative research grant from British Council, Tokyo, Japan.*

## REFERENCES

Bhogal B, Wojnarowska F, Marsden RA, Das A, Black MM, McKee PH: Linear IgA bullous dermatosis of adults and children: an immunoelectron microscopic study. *Br J Dermatol* 117:289–296, 1987

Burge S, Wojnarowska F, Marsden A. Chronic bullous dermatosis of childhood persisting into adulthood. *Pediatr Dermatol* 5:246–249, 1988

Chorzelski TP, Jablonska S, Beutner EH, Wilson BD: Linear IgA bullous dermatosis. In: Beutner EH, Chorzelski TP, Kumar V (eds.). *Immunopathology of the Skin.* Wiley Medical Publications, New York, 1987, pp 407–420

Dabrowski J, Chorzelski TP, Jablonska S, Krainska T, Jarzabek-Chorzelska M: The ultrastructural localization of IgA in skin of a patient with mixed form of dermatitis herpetiformis and bullous pemphigoid. *J Invest Dermatol* 70:76–79, 1978

Dabrowski J, Chorzelski TP, Jablonska S, Krainska T, Jarzabek-Chorzelska M: The ultrastructural localization of IgA deposits in chronic bullous disease of childhood (CBDC). *J Invest Dermatol* 71:291–295, 1979

Dmochowski M, Hashimoto T, Bhogal BS, Black MM, Zone JJ, Nishikawa T: Immunoblotting studies of linear IgA disease. *J Dermatol Sci* 6:194–200, 1993

Fine JD, Horiguchi Y, Couchman JR: 19-DEJ-1, a hemidesmosome-anchoring filament complex-associated monoclonal antibody. Definition of a new skin basement membrane antigenic defect in junctional and dystrophic epidermolysis bullosa. *Arch Dermatol* 125:520–523, 1989

Gammon WR, Briggaman RA, Inman AO, Queen LL, Wheeler CE: Differentiating anti-lamina lucida and anti-sublamina densa anti-BMZ antibodies by indirect immunofluorescence on 1.0M sodium chloride separated skin. *J Invest Dermatol* 82:139–144, 1984

Haftek M, Zone JJ, Taylor TB, Kowalewski C, Chorzelski TP, Schmitt D: Immunogold localization of the 97-kD antigen of linear IgA bullous dermatosis (LABD) detected with patients' sera. *J Invest Dermatol* 103:656–659, 1994

Horiguchi Y, Toda K, Okamoto H, Imamura S: Immunoelectron microscopic observation in a case of linear IgA bullous dermatosis of childhood. *J Am Acad Dermatol* 14:593–599, 1986

Ishiko A, Shimizu H, Kikuchi A, Ebihara T, Hashimoto T, Nishikawa T: Human autoantibodies against the 230-kD bullous pemphigoid antigen (BPAG1) bind only to the intracellular domain of the hemidesmosome, whereas those against the 180-kD bullous pemphigoid antigen (BPAG2) bind along the plasma membrane of the hemidesmosome in normal human and swine skin. *J Clin Invest* 91:1608–1615, 1993

Karpati S, Stolz W, Meurer M, Krieg T, Braun-Falco O: Ultrastructural immunogold studies in two cases of linear IgA dermatosis. Are there two distinct types of this disease? *Br J Dermatol* 127:112–118, 1992

Lawley TJ, Strober W, Yaoita H, Katz SI: Small intestinal biopsies and HLA types in dermatitis herpetiformis patients with granular and linear IgA skin deposits. *J Invest Dermatol* 74:9–12, 1980

Legrain V, Taieb A, Surleve-Bazeille JE, Bernard P, Maleville J: Linear IgA dermatosis of childhood: case report with an immunoelectron microscopic study. *Pediatr Dermatol* 8:310–313, 1991

Onodera Y, Hashimoto T, Miyakawa S, Shimizu H, Nishikawa T: A case of linear IgA bullous dermatosis of childhood: immunoelectron microscopic and IgA subclass studies. *Dermatologica* 180:267–271, 1990

Pehamberger H, Konrad K, Holubar K: Circulating IgA anti-basement membrane antibodies in linear dermatitis herpetiformis (Duhring): immunofluorescence and immunoelectronmicroscopic studies. *J Invest Dermatol* 69:490–493, 1977

Petersen MJ, Gammon WR, Briggaman RA: A case of linear IgA disease presenting initially with IgG immune deposits. *J Am Acad Dermatol* 14:1014–1019, 1986

Prost C, De Leca AC, Combemale P, Labeille B, Martin N, Cosnes A, Guillaume JC, Venencie PY, Verret JL, Dubertret L, Touraine R: Diagnosis of adult linear IgA dermatosis by immunoelectronmicroscopy in 16 patients with linear IgA deposits. *J Invest Dermatol* 92:39–45, 1989

Shimizu H, McDonald AR, Kennedy AR, Eady RAJ: Demonstration of intra- and extracellular localization of bullous pemphigoid antigen using cryofixation and freeze substitution for postembedding immunoelectron microscopy. *Arch Dermatol Res* 281:443–448, 1989

Shimizu H, Ishida-Yamamoto A, Eady RAJ: The use of 1-nm gold probes for light and electron microscopic localization of intra- and extracellular antigens in skin. *J Hictochem Cytochem* 40:883–888, 1992

Tokuyasu KT: Application of cryoultramicrotomy to immunocytochemistry. J Microsc 143:139–149, 1985

Verrando P, Blanchet BC, Pisani A, Thomas L, Cambazard F, Eady RAJ, Schofield O, Ortonne JP: Monoclonal antibody GB3 defines a widespread defect of several basement membranes and a keratinocyte dysfunction in patients with lethal junctional epidermolysis bullosa. *Lab Invest* 64:85–92, 1991

Willsteed E, Bhogal BS, Black MM, McKee P, Wojnarowska F: Use of 1M NaCl split skin in the inderect immunofluorescence of the linear IgA bullous dermatoses. *J Cutan Pathol* 17:144–148, 1990

Wojnarowska F, Marsden RA, Bhogal B, Black MM: Chronic bullous disease of childhood, childhood cicatricial pemphigoid, and linear IgA disease of adults. A comparative study demonstrating clinical and immunopathologic overlap. *J Am Acad Dermatol* 19:792–805, 1988

Wojnarowska F, Whitehead P, Leigh IM, Bhogal BS, Black MM: Identification of the target antigen in chronic bullous disease of childhood and linear IgA disease of adults. *Br J Dermatol* 124:157–162, 1991

Wojnarowska F, Collier PM, Allen J, Millard PR: The localization of the target antigens and antibodies in linear IgA disease is heterogeneous, and dependent on the methods used. *Br J Dermatol* 132:750–757, 1995

Yamasaki Y, Hashimoto T, Nishikawa T: Dermatitis herpetiformis with linear IgA deposition: ultrastructural localization of in vivo bound IgA. *Acta Derm Venereol (Stockh)* 62:401–405, 1982

Yaoita H, Katz SI: Immunoelectronmicroscopic localization of IgA in skin of patients with dermatitis herpetiformis. *J Invest Dermatol* 67:502–506, 1976

Yaoita H, Katz SI: Circulating IgA anti-basement membrane zone antibodies in dermatitis herpetiformis. *J Invest Dermatol* 69:558–560, 1977

Zone JJ, Taylor TB, Kadunce DP, Meyer LJ: Identification of the cutaneous basement membrane zone antigen and isolation of antibody in linear immunoglobulin A bullous dermatosis. *J Clin Invest* 85:821–820, 1990